Symposium: Diet, Anthropometry and Breast Cancer: Integration of Experimental and Epidemiologic Approaches

Genetic Factors in the Pathogenesis of Breast Cancer: Their Role and Relative Importance[1,2]

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ABSTRACT Aggregation of breast cancer in families is an established risk factor associated with increased incidence of the disease, which is a leading cause of morbidity and mortality among women in this country. Three genes have now been identified that confer increased susceptibility in families with a clear hereditary (i.e., Mendelian) pattern of expression: *BRCA1*, *BRCA2* and *p53*. However, a significant number of women have an identified family history of breast cancer without clear Mendelian patterns of disease. Such patterns are consistent with the effect of more common genes with lower associated risk. Some evidence is available to implicate three additional genes that fit this category: *AT*, *ESR* and *HRAS1*. An area of active interest is genetically mediated variation in the metabolism of estrogens, a process controlled by several genes, each with more modest effects. The interaction of genes and environmental factors in breast cancer pathogenesis is of considerable public health importance. J. Nutr. 127: 929S–932S, 1997.

KEY WORDS: • *breast cancer* • *genes* • *risk factors* • *nutrition* • *review*

It has been firmly established that at least a portion of the occurrence of breast cancer can be ascribed to inherited susceptibility. There are currently three genes (*p53*, *BRCA1* and *BRCA2*) known that, when inherited in an altered form, confer very high lifetime risks (high penetrance in genetic terms) and high relative risks (in epidemiologic terms). Families segregating a mutation in one of these genes demonstrate an autosomal dominant pattern of susceptibility. These genes seem to be an infrequent cause of breast cancer in the general population and are associated primarily with early-onset breast cancer (Ford and Easton 1995).

p53

Inherited mutations in the *p53* gene seem to be responsible for some (Malkin et al. 1990) but not all (Santibanez-Koref et al. 1991) cases of the Li-Fraumeni syndrome. However, this syndrome is rare in the population and has been estimated to account for less than 1% of cases of breast cancer, even at young ages (Sidransky et al. 1992).

BRCA1

The *BRCA1* gene was initially localized to chromosome 17q21 in families with breast-ovarian cancer and early-onset breast cancer (Hall et al. 1990) and was recently cloned (Miki et al. 1994). Genetic linkage studies have shown that almost all multiple-case breast-ovarian cancer families are counted segregating mutations in *BRCA1* (Narod et al. 1995). At age 40 y, women who inherit a mutation in *BRCA1* are at roughly 200-fold greater risk of breast cancer than the general population. This risk decreases somewhat with age, but remains elevated roughly 15-fold to age 70 y. Ford et al. (1995) used two population-based samples of families and estimated the frequency of *BRCA1* mutations to be 0.0006 [95% confidence interval (CI) 0.0002 to 0.001] and the proportion of breast cancer cases due to *BRCA1* to be 5.3% below age 40 y, 2.2% between ages 40 and 49 y, and 1.1% between ages 50 and 70 y. They concluded that "the majority of breast cancer families with less than four cases and no ovarian cancer are not due to rare highly penetrant genes such as *BRCA1* but are more likely to be due to either chance or to more common genes of lower penetrance." Langston et al. (1996) studied 80 women with onset of breast cancer before the age of 35 y and found mutations in eight and alterations of unknown functional importance in another four. Intriguingly, two of the six women with mutations did not report a family history of breast or ovarian cancer in first- or second-degree relatives, but the number of relatives at risk and their ages were not presented. Fitzgerald et al. (1996) obtained similar estimates of alterations in a sample of 30 women with breast cancer onset before age 30 y. The complete phenotype associated with mutations in

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this gene is not known, but a recent report of 33 families with evidence for linkage to *BRCA1* revealed statistically significant excesses of colon cancer and prostate cancer but no deficits or excesses for any other sites (Ford et al. 1994).

BRCA2

Because *BRCA1* was observed to account for only roughly 40% of the identified families with apparent autosomal dominant patterns of breast cancer, the search for other genes in these families continued. *BRCA2* was localized to chromosome 13q and recently cloned (Wooster et al. 1995). Like *BRCA1*, *BRCA2* is thought to account for approximately 40% of the families with apparent hereditary predisposition to breast cancer and to confer a high risk of early-onset breast cancer in females. Although the gene was localized in families that also had cases of male breast cancer, affected males are also observed in families with *BRCA1* mutations. Analysis of different tumor types from five families showing evidence of linkage to *BRCA2* revealed loss of heterozygosity of the wild-type chromosomal region in tumors of the prostate, ovary, cervix, colon and ureter (Gudmundsson et al. 1995). This information suggests a broader phenotype may be more appropriate for *BRCA2* than *BRCA1*.

All three genes (*p53*, *BRCA1* and *BRCA2*) are thought to be tumor suppressor genes. In the families studied to date, there is a high probability that members who inherit a mutation develop cancer, but the frequencies of inherited mutations in the general population seem to be low. For *BRCA1* and *BRCA2*, the frequency of mutation carriers in the United States is estimated to be on the order of one in 400 (Ford et al. 1995), or roughly 300,000 women. Thus, factors that influence penetrance of the gene need to be identified. In particular, those factors that prevent elderly mutation carriers from developing cancer despite their inherited high predisposition might prove to be equally important and relevant to women without such strong susceptibility. The role of environmental factors, especially modifiable ones such as diet, is an important area of research that needs to be addressed.

GENETIC FACTORS AND NON-MENDELIAN FAMILIAL BREAST CANCER

Although the importance of the discoveries of major susceptibility genes for breast cancer cannot be minimized, the public health significance remains to be determined (Fitzgerald et al. 1996, Langston et al. 1996). In particular, according to data from the Surveillance, Epidemiology and End Results program, fully 80% of all breast cancers occur in women over the age of 55 y (National Cancer Institute 1990). Given that late-onset breast cancer consistently demonstrates familial aggregation and that the clustering does not seem to be explained by chance or measured nongenetic risk factors (Chen et al. 1994), it is reasonable to hypothesize that genes other than *BRCA1*, *BRCA2* and *p53* might be contributing factors. In fact, there are at least three genes (*AT*, *ESR*, *HRAS1*) for which there is some evidence for an association with breast cancer. Variations in these genes are characterized as having low penetrance and low relative risks and might be involved in the non-Mendelian familial clustering of breast cancer that occurs at more typical ages at onset. In addition, there is evidence that variations or alterations in these genes are more common in the general population than mutations in *BRCA1*, *BRCA2* or *p53*. Even with the associated lower relative risks, however, the total number of cases in the population attributable to inherited alterations in these genes may be considerable.

ATAXIA TELANGIECTASIA

Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by cerebellar ataxia, oculocutaneous telangiectasia, a hypersensitivity to ionizing radiation and an increased susceptibility to cancer (McKinnon 1987). Four studies have shown that female relatives of AT patients (obligate gene carriers) are at increased risk of breast cancer. A review of these studies (Easton 1994) produced a combined estimate of relative risk of breast cancer associated with AT heterozygosity of 3.9 (95% CI 2.1 to 7.2). It is important to verify these observations. Because the AT gene has been cloned (Savitsky et al. 1995), it is now possible to base exposure classification on genotype data and extend the study samples to women outside of AT families. It is relevant to note that a relative risk of this magnitude is unlikely to yield multiple cases of early-onset breast cancer in a family in a pattern consistent with simple Mendelian segregation. Wooster et al. (1993) studied 16 multiplex breast cancer families and found no evidence for genetic linkage between breast cancer and chromosome 11q markers (the physical location of the AT gene). Although AT patients are known to have radiosensitivity, they are also more susceptible to cell damage from hydroxy radicals. Thus, it would seem appropriate to investigate the risk of breast cancer among women who carry an altered AT gene and who have low dietary exposure to antioxidants; susceptible individuals may not develop breast cancer unless they receive relevant exposure.

HRAS1

The proto-oncogene *HRAS1* is tightly genetically linked to a minisatellite locus that is highly polymorphic. Of the 30 or so alleles that have been identified, four common alleles account for 94% of all alleles in white persons (Krontiris 1990). A meta-analysis of 23 studies has shown that rare *HRAS1* alleles are associated with a 1.9-fold (95% CI 1.6 to 2.3) increased risk of cancer (Krontiris et al. 1993). Statistically significant elevated risk estimates were evident for cancers of the breast (1.7), bladder (2.3), colon/rectum (2.2) and lung (1.6) and for leukemia (2.3). Just as for AT, these magnitudes of relative risk are probably insufficient for hereditary manifestation of susceptibility. Indeed, Hall et al. (1990) found no evidence of linkage of *HRAS1* to breast cancer susceptibility in 12 high risk families with a Mendelian pattern of disease. Because of the relative high frequency of the rare alleles, however, Krontiris et al. (1993) estimated that as many as one in 11 cancers may be attributed to this factor. Because the biologic mechanism underlying the apparent increased breast cancer risk among women carrying rare *HRAS1* alleles is unknown, examination of possible interactions of rare *HRAS1* alleles with aspects of diet is perhaps premature at this time.

ESTROGEN RECEPTOR

The data that directly link estrogens to breast cancer risk include animal models and studies of breast tumor cell lines; the human data are largely indirect, but strong. The biologic role of estrogens is mediated through high affinity binding to the estrogen receptor (ESR), a member of a family of ligand-inducible nuclear receptors that have steroid and thyroid hormones and vitamins as known ligands (Evans 1988). At least three published studies implicate the ESR in familial breast cancer. Zuppan et al. (1991) reported genetic linkage of the ESR locus with late-onset breast cancer, but the results were not statistically significant. Andersen et al. (1994) compared

genomic and tumor DNA of 274 breast cancer patients with DNA from 204 controls for restriction fragment length polymorphisms and found suggestive evidence that the frequency of the shorter alleles was 1.4 times higher among breast cancer patients (95% CI 1.03 to 1.85). These alleles tended to be associated with later age at onset, but the results were not statistically significant. Roodi et al. (1995) examined the entire coding region of the ESR gene in 118 receptor-positive and 70 receptor-negative breast cancers. Mutations were observed in only two cases (1%), but several neutral polymorphisms (i.e., base pair substitutions that did not alter the encoded amino acid) were identified. Intriguingly, the polymorphism at codon 325 was strongly associated ($P < 0.0005$) with a positive family history of breast cancer. The fact that certain vitamins act as ligands to the ESR suggests that simultaneous consideration of dietary intake and genetic constitution could be informative in further elucidation of the role of the ESR gene.

ESTROGEN METABOLISM

The etiologic significance of hormones in the pathogenesis of breast cancer is generally accepted (Henderson and Bernstein 1996). It has been repeatedly shown that estrogens can induce and promote mammary tumors in rodents (Bernstein 1993). Epidemiologic studies have consistently demonstrated that early menarche, late menopause, nulliparity, late age at first full-term pregnancy, and obesity in postmenopausal women are associated with significant excess risks of breast cancer (Henderson and Bernstein 1996). These risk factors are thought to exert their effect via longer periods of exposure to estrogens secreted by the ovaries.

The metabolism of estrogen is controlled by several enzymes, each of which is encoded by separate genes. Most estradiol (E_2) is metabolized, first to estrone (E_1) and subsequently to either 16α-hydroxyestrone (16α-OHE_1) or 2-hydroxyestrone (2-OHE_1) (Martucci and Fishman 1993). The latter exhibits virtually no peripheral estrogenic effect and in fact may act as an anti-estrogen in estrogen-sensitive tissues (Schneider et al. 1984). The former, however, retains uterotropic activity (Yu and Fishman 1985) and, unlike the parent estrogen E_2, possesses initiator and promoter activity in normal mammary epithelial cells (Telang et al. 1992). It has been shown that 16α-OHE_1 can covalently bind to the estrogen receptor and produce prolonged biochemical or physiological response in animals (Bradlow et al. 1992). Laboratory experiments have shown that 16α-OHE_1 is elevated in explant cultures of mammary terminal duct lobular units of breast cancer patients (Osborne et al. 1993). Epidemiologic studies in humans have indicated that breast cancer patients have substantially lower 2- to 16α-OHE_1 ratios than controls (Schneider et al. 1982). Taken together, these data suggest that genetic variation in enzyme activity may be related to breast cancer risk.

In addition to estrone 2- and 16α-hydroxylases, other enzymes (e.g., 17-hydroxysteroid dehydrogenase, aromatase) are also directly involved in estrogen synthesis and clearance. Many proteins or peptides are involved in estrogen regulation (e.g., follicle-stimulating hormone and luteinizing hormone) and transport (e.g., sex hormone–binding globulin), and these compounds are in turn regulated by other factors. It is certain that the complex process of estrogen regulation, synthesis, transport and clearance is determined, to a large extent, by genetic factors. Therefore, it is conceivable that endogenous estrogens and their metabolism patterns may explain interindividual variation in risk of breast cancer. This hypothesis, however, has not been adequately investigated.

LITERATURE CITED

Andersen, T. I., Heimdal, K. R., Skrede, M., Tveit, K., Berg, K. & Børresen, A.-L. (1994) Oestrogen receptor (ESR) polymorphisms and breast cancer susceptibility. Hum. Genet. 94: 665–670.

Bernstein, L. (1993) Endogenous hormones and breast cancer risk. Epidemiol. Rev. 15: 48–65.

Bradlow, H. L., Michnovicz, J. J., Telang, N., Osborne, M. P. & Goldin, B. R. (1992) Diet, oncogenes and tumor viruses as modulators of estrogen metabolism *in vivo* and *in vitro*. Cancer Detect. Prev. 16: S35–S41.

Chen, P.-L., Sellers, T. A., Rich, S. S., Potter, J. D. & Folsom A. R. (1994) Examination of the effect of non-genetic risk factors on the familial risk of breast cancer among relatives of postmenopausal breast cancer patients. Cancer Epidemiol. Biomarkers Prev. 3: 549–556.

Easton, D. F. (1994) Cancer risks in A-T heterozygotes. Int. J. Radiat. Biol. 66: S177–S182.

Evans, R. M. (1988) The steroid and thyroid hormone receptor superfamily. Science 240: 889–895.

Fitzgerald, M. G., MacDonald, D. J., Krainer, M., Hoover, I., O'Neil, E., Unsal, H., Silva-Arrieto, S., Finkelstein, D. M., Beer-Romero, P., Englert, C., Sgroi, D. C., Smith, B. L., Younger, J. W., Garber, J. E., Duda, R. B., Mayzel, K. A., Isselbacher, K. J., Friend, S. H. & Haber, D. A. (1996) Germ-line BRCA1 mutations in Jewish and non-Jewish women with early-onset breast cancer. N. Engl. J. Med. 334: 143–149.

Ford, D. & Easton, D. F. (1995) The genetics of breast and ovarian cancer. Br. J. Cancer 72: 805–812.

Ford, D., Easton, D. F., Bishop, D. T., Narod, S. A. & Goldgar, D. E. (1994) Risks of cancer in BRCA1 mutation carriers. Lancet 343: 692–695.

Ford, D., Easton, D. F. & Peto, J. (1995) Estimates of the gene frequency of BRCA1 and its contribution to breast and ovarian cancer incidence. Am. J. Hum. Genet. 57: 1457–1462.

Gudmundsson, J., Johannesdottir, G., Bergthorsson, J. T., Arason, A., Ingvarsson, S., Egilsson, V. & Barkardottir, R. B. (1995) Different tumor types from BRCA2 carriers show wild-type chromosome deletions on 13q12-q13. Cancer Res. 55: 4830–4832.

Hall, J. M., Huey, B., Morrow, J., Newman, B., Lee, M., Jones, E., Carter, C., Buehring, G. C. & King, M.-C. (1990) Rare HRAS alleles and susceptibility to human breast cancer. Genomics 6: 188–191.

Henderson, B. E. & Bernstein, L. (1996) Endogenous and exogenous hormone factors. In: Diseases of the Breast (Harris, J. R., Lippman, M. E., Morrow, M. & Hellman, S., eds.), pp. 185–200. Lippincott-Raven Publishers, Philadelphia, PA.

Krontiris, T. G. (1990) Detection of cancer predisposition by hypervariable region analysis. Birth defects: original article series. J. Cell. Biochem. 26: 129–140.

Krontiris, T. G., Devlin, B., Karp, D. D., Robert, J. J. & Risch, N. (1993) An association between the risk of cancer and mutations in the HRAS1 minisatellite locus. N. Engl. J. Med. 329: 517–523.

Langston, A. A., Malone, K. E., Thompson, J. D., Daling, J. R. & Ostrander E. A. (1996) BRCA1 mutations in a population-based sample of young women with breast cancer. N. Engl. J. Med. 334: 137–142.

Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Jr., Nelson, C. E., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z., Tainsky, M. A. & Friend S. H. (1990) Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250: 1233–1238.

Martucci, C. P. & Fishman, J. (1993) P450 enzymes of estrogen metabolism. Pharmacol. & Ther. 57: 237–257.

McKinnon, P. J. (1987) Ataxia-telangiectasia: an inherited disorder of ionizing-radiation sensitivity in man. Progress in the elucidation of the underlying biochemical defect. Hum. Genet. 75: 197–208.

Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L. M., Ding, W., Bell, R., Rosenthal, J., Hussey, C., Tran, T., McClure, M., Frye, C., Hattier, T., Phelps, R., Haugen-Strano, A., Katcher, H., Yakumo, K., Gholami, Z., Shaffer, D., Stone, S., Bayer, S., Wray, C., Bogden, R., Dayananth, P., Ward, J., Tonin, P., Narod, S., Bristow, P. K., Norris, F. H., Helvering, L., Morrison, P., Rosteck, P., Lai, M., Barrett, J. C., Lewis, C., Neuhausen, S., Cannon-Albright, L., Goldgar, D., Wiseman, R., Kamb, A. & Skolnick, M. H. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66–71.

Narod, S., Ford, D., Devilee, P., Barkardottir, R. B., Eyfjord, J., Lenoir, G., Serova, O., Easton, D. & Goldgar D. (1995) Genetic heterogeneity of breast-ovarian cancer revisited. Breast Cancer Linkage Consortium. Am. J. Hum. Genet. 57: 957–958.

National Cancer Institute SEER Program (1990) Cancer Statistics Review, 1973–1987. NIH Publication No. 90-2789. National Institute of Health, Bethesda, MD.

Osborne, M. P., Bradlow, H. L., Wong, G.Y.C. & Telang, N. T. (1993) Upregulation of estradiol C16α-hydroxylation in human breast tissue: a potential biomarker of breast cancer risk. J. Natl. Cancer Instit. 85: 1917–1920.

Roodi, N., Bailey, L. R., Kao, W.-Y., Verrier, C. S., Yee, C. J., Dupont, W. D. & Parl, F. F. (1995) Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. J. Natl. Cancer Instit. 87: 446–451.

Santibanez-Koref, M. F., Birch, J. M., Hartley, A. L., Jones, P. H., Craft, A. W.,

Eden, T., Crowther, D., Kelsey, A. M. & Harris, M. (1991) p53 germline mutations in Li-Fraumeni syndrome. Lancet 338: 1490–1491.

Savitsky, K., Bar-Shira, A., Gilad, S., Rotman, G., Ziv, Y., Vanagaite, L., Tagle, D. A., Smith, S., Uziel, T., Sfez, S., Ashkenazi, M., Pecker, I., Frydman, M., Harnik, R., Patanjali, S. R., Simmons, A., Clines, G. A., Sartiel, A., Gatti, R. A., Chessa, L., Sanal, O., Lavin, M. F., Jaspers, N.G.J., Taylor, M. R., Arlett, C. F., Miki, T., Weissman, S. M., Lovett, M., Collins, F. S. & Shiloh, Y. (1995) A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 268: 1749–1753.

Schneider, J., Huh, M. M., Bradlow, H. L. & Fishman, J. (1984) Antiestrogen action of 2-hydroxyestrone on MCF-7 human breast cancer cells. J. Biol. Chem. 259: 4840–4845.

Schneider, J., Kinne, D., Fracchia, A., Pierce, V., Anderson, K. E., Bradlow, H. L. & Fishman, J. (1982) Abnormal oxidative metabolism of estradiol in women with breast cancer. Proc. Natl. Acad. Sci. U.S.A. 79: 3047–3051.

Sidransky, D., Tokino, T., Helzlsourer, K., Zehnbauer, B., Rausch, G., Shelton, B., Prestigiacomo, L., Vogelstein, B. & Davidson, N. (1992) Inherited p53 gene mutations in breast cancer. Cancer Res. 52: 2984–2985.

Telang, N. T., Osborne, M. P., Sweterlitsch, L. A., Narayanan, R. (1992) Neoplastic transformation of mouse mammary epithelial cells by deregulated myc expression. Cell Regul. 1: 863–872.

Wooster R., Ford D., Mangion J., Ponder B. A., Peto J., Easton D. F. & Stratton, M. R. (1993) Absence of linkage to the ataxia telangiectasia locus in familial breast cancer. Hum. Genet. 92: 91–94.

Wooster, R., Bignell, G., Lancaster, J., Swift, S., Seal, S., Mangion, J., Collins, N., Gregory, S., Gumbs, C., Micklem, G., Barfoot, R., Hamoudi, R., Patel, S., Rice, C., Biggs, P., Hashim, Y., Smith, A., Connor, F., Arason, A., Gudmundsson, J., Ficenec, D., Kelsell, D., Ford, D., Tonin, P., Bishop, D. T., Spurr, N. K., Ponder, B.A.J., Eeles, R., Peto, J., Devilee, P., Cornelisse, C., Lynch, H., Narod, S., Lenoir, G., Eglisson, V., Barkadottir, R. B., Easton, D. F., Bentley, D. R., Futreal, P. A., Ashworth, A. & Stratton, M. R. (1995) Identification of the breast cancer susceptibility gene BRAC2. Nature (Lond.) 378: 789–792.

Yu, S. & Fishman, J. (1985) Interactions of histones with estrogens. Covalent adduct formation with 16α-hydroxyestrone. Biochemistry 29: 8017–8021.

Zuppan, P., Hall, J. M., Lee, M. K., Pongiikitmongkol, M. & King M.-C. (1991) Possible linkage of the estrogen receptor gene to breast cancer in a family with late-onset disease. Am. J. Hum. Genet. 48: 1065–1068.